[0221] Fabrication of Multi-Layered Vascular Tubes

[0222] Preparation of NovoGel™ base plate: A NovoGel™ base plate was fabricated by dispensing 10 ml of pre-warmed (>40° C.) NovoGel™ (2% w/v) into a 10 cm Petri dish. Immediately after dispensing, the NovoGel™ was evenly spread so as to cover the entire base of the dish and form a uniform layer. The Petri dish was incubated at room temperature for 20 minutes to allow the NovoGel™ to gel completely.

[0223] Multi-layered vascular tube: Vascular tubes consisting of an outer layer of HDF and an inner layer of HASMC-HAEC were fabricated utilizing HDF cylinders, and HASMC-HAEC mixed cell cylinders. A geometrical arrangement as shown in FIG. **4** was utilized. Briefly, at the end of the 24-hour incubation period mature HDF and HASMC-HAEC cylinders were aspirated back into the capillary tubes and placed in appropriate culture medium until further use. The support structure consisting of NovoGel™ rods was prepared as follows: Pre-warmed 2% NovoGel™ was aspirated into the capillary tubes (L=50 mm) and rapidly cooled in cold PBS solution (4° C.). The 5 cm long gelled NovoGel™ cylinder was deposited from the capillary (using the plunger) and laid down straight on the NovoGel™ base plate. A second NovoGel™ cylinder was adjoined to the first one and the process was repeated until 10 NovoGel™ cylinders were deposited to form the first layer. At this point 20 μl of PBS was dispensed above the NovoGel™ cylinders to keep them wet. Further six NovoGel™ cylinders were deposited on top of layer 1 at positions as shown in FIG. **4** (layer 2). Three HDF cylinders were then deposited at positions 4, 5 and 6 to complete layer 2. After dispensing each HDF cylinder 40 μl of HDF culture medium was dispensed on top of the deposited cylinder to assist the deposition of the subsequent cylinder as well as to prevent dehydration of the cellular cylinders. Next NovoGel™ cylinders for layer 3 were deposited followed by HDF cylinders at positions 3 and 6. Following rewetting of the structure with HDF culture medium, HASMC-HAEC mixed cylinders were laid down in positions 4 and 5. Subsequently, 40 μl of HASMC medium and 40 μl of HDF medium were dispensed on top of the cell cylinders. Layer 4 was completed by depositing NovoGel™ cylinders at positions 1 and 7, HDF cylinders at positions 2 and 6, HASMC-HAEC mixed cylinders at positions 3 and 5, and finally a 4% NovoGel™ cylinder at position 4. Layers 5, 6 and 7 were completed similarly by laying down NovoGel™ cylinders followed by HDF cylinders and finally HASMC-HAEC cylinders at positions shown in FIG. **4**. Once the entire construct was completed 0.5 ml of warm NovoGel™ was dispensed over each end of the construct and allowed to gel at room temperature for 5 minutes. Following gelation of that NovoGel™, 30 ml of HASMC medium was added to the Petri dish (to ensure the entire construct was completely submerged). The construct was incubated for 24 hours at 37° C. and 5% CO_2 to allow for fusion between the cellular cylinders.

[0224] At the end of 24 hours, the surrounding NovoGel™ support structure was removed from the fused multi-layered vascular tube.

Example 3: Bioprinter

[0225] A bioprinter was assembled. The bioprinter contained a printer head having a collet chuck grip for holding a cartridge, and a piston for dispensing the contents of the cartridge. The cartridges used were glass microcapillary tubes having a length of 75-85 mm. A new capillary tube was loaded each time bio-ink or support material was required.

[0226] In order to print structures, a dispense position repeatability of ±20 μm was required for the duration of the printing process, i.e., when new capillaries were loaded into the printer head. In order to maintain repeatability of all loaded capillary tubes relative to the same point in the x-, y-, and z-directions, the bioprinter contained a laser calibration system for calibrating the position of the microcapillary tube. The laser calibration system calibrated the position of all capillary tips to a common reference location. All printing moves were made relative to this reference position.

[0227] All three axes (x-, y-, and z-axes) were calibrated through usage of a single laser distance measurement sensor. The system consisted of a laser sensor and a laser beam. The sensor threshold was the maximum sensing distance of the laser sensor. The sensor was configured to ignore all signals further away than a pre-defined threshold. The sensor used triangulation to determine distance to the object (the capillary tip). The laser sensor was orientated with the beam aimed vertically up (+z-axis).

[0228] Vertical Laser Calibration

[0229] For calibration in the x-axis: The capillary tip was moved in the range of the laser sensor, with the tip to the left (−x) of the laser beam. The capillary was moved to in the +x direction until the sensor detected the capillary edge, and this position was recorded. The above steps were repeated from the opposite side (i.e., the tip was positioned at the right (+x) of the laser beam and moved in the −x direction until the sensor detected the capillary edge). The positions from both steps were averaged to calculate the mid-point of the capillary. Optionally, the above process was repeated for different y-positions and the calculated mid-points were averaged.

[0230] For calibration in the y-axis: The above procedure (for the x-axis) was repeated for the y-axis.

[0231] For calibration in the z-axis: The capillary tip was moved to above the sensor beam so that the bean hit the bottom surface of the capillary, and the tip was just outside of the sensor range threshold. The capillary was lowered until the sensor threshold was reached, and that position was recorded as the z-position. Optionally, the above steps were repeated at multiple points on the capillary tip surface and measured heights were averaged.

[0232] Horizontal Laser Calibration

[0233] For calibration in the y-axis: The capillary was moved so that the tip was just below the laser beam height, and the capillary was off to one side (in the y-direction). The capillary was moved in the y-direction towards the laser. The capillary was stopped when the laser sensor detected the beam reflected off the capillary, and this position was recorded. The above steps were repeated with the capillary off to the other side of the laser, and moved in the −y direction). The mid-point from the above steps was recorded as the y-position.

[0234] For calibration in the x-axis: Using the results of the calibration in the y-axis, the y-axis was moved so that the laser was centered on the capillary. The capillary was moved past the sensor threshold and moved towards the sensor. The capillary was stopped as soon as the capillary crossed the sensor threshold and the sensor output changed. This position, plus ½ the capillary width (from the y-calibration) was recorded as the x-position.